REMARKS

Reconsideration of this application is requested.

Claims 29-32 have been rejected on formal and obviousness grounds for the reasons set forth throughout the action. In response to those rejections, all of the claims in this application have been cancelled and replaced by new claims 33-36. For the reasons discussed below, it is believed that the new claims presented with this response obviate the outstanding formal rejection, and also define subject matter which is clearly patentably distinguished over the cited art. Withdrawal of all of the outstanding rejections is therefore respectfully requested.

Claims 29-32 have been rejected under 35 USC 112, first paragraph, on the ground that the disclosure is enabling only for claims limited to essentially full sequence factor IX protein. In response to that rejection, it will be seen that new independent claims 33 and 35 specify that the factor IX protein "essentially has the amino acid sequence of human factor IX protein", in accordance with the Examiner's observation. Thus, the language "or of a protein sufficiently similar thereto to make it acceptable for infusion into human patients suffering from factor IX deficiency" appearing in the previous claims does not appear in new claims 33 and 35. It is believed therefore that new claims 33 and 35 are fully enabled by the specification. Withdrawal of the formal rejection is accordingly respectfully requested.

Claims 29 through 32 have been rejected under 35 USC 103 as being unpatentable over Suomela et al (hereinafter Suomela) or Osterud et al (hereinafter Osterud) when taken in view of Schwinn. That rejection is traversed for the following reasons.

As now claimed, the invention is directed to a plasma-free preparation suitable for use in the treatment of human patients suffering from deficiency of factor IX. The preparation comprises, as active ingredient, biologically active recombinant DNA-derived factor IX protein derived from a single human individual and which (1) essentially has the amino acid sequence of human factor IX protein, (2) is free from contamination by poxviruses and by all plasma constituents, and (3) has a specific activity (as defined in claim 33) of at least 90% of that of average normal human plasma. The invention also provides a method of treating human patients suffering from deficiency of factor IX, in which a plasma-free preparation as defined in claim 33 is administered to the patient.

The expression "plasma-free preparation" finds support in the application as originally filed in the last sentence appearing on page 31. The introductory language of new method claim 35 is in line with the field of use statement appearing in claim 33.

In discussing the pertinence of the applied art, it is important to recognize the various specifically recited features characterizing the claimed preparation and method of the present invention. Thus, the claimed preparation is:

(i) plasma-free;

(ii) suitable for use in the treatment of human patients suffering from deficiency of factor IX;

(iii) biologically active recombinant DNA-derived factor IX protein;

(iv) derived from a single human individual and essentially has the amino acid sequence of human factor IX protein; and

(v) is free from contamination by poxviruses and all plasma constituents and has a defined specific activity of at least 90% of that of average normal human plasma.

As noted in the introductory part of the specification, injection of factor IX concentrate obtained from blood donors allows most patients suffering from hemophilia B or Christmas disease to be adequately treated. However, this treatment involves some risk of infection by blood-borne viruses due to impurities in the factor IX concentrate presently in use. It is important, therefore, to employ a factor IX protein obtained from a source **other than blood**.

As is clear from the description appearing at page 1 beginning at line 20 through page 4 line 24, extensive work has been carried out in the past directed to the preparation of factor IX protein. However, as noted in that discussion, difficulties have been continually encountered in obtaining a fully biologically active factor IX protein. Moreover, the method suggested by Transgene (see the paragraph bridging pages 3 and 4 of the present application) involves expression of

factor IX protein in vaccinia virus and cowpox virus. That method not only results in a protein having about 50% of the biologically active normal plasma factor IX, but also gives rise to a more serious problem in that the factor IX protein thus produced requires rigorous purification to remove live poxviral particles and antigens from the dead poxvirus, as well as removal of any vaccinia virus remaining in the product.

The present inventors have discovered, surprisingly, that it is possible to produce an artificial fully or near fully biologically active factor IX protein using recombinant DNA technology in mammalian cells without recourse to poxvirus vectors. Thus, the factor IX protein product of the present invention is plasma-free, essentially has the amino acid sequence of human factor IX protein and is free from contamination by poxviruses and other plasma constituents, thereby making the present product ideal for use in the treatment of human patients suffering from deficiency of factor IX.

Osterud describes a crude purification method based on chromatography of plasma material on a heparin column.

Schwinn describes a procedure in which a crude concentrate from plasma is heated with a calcium salt, glycine and sucrose to remove hepatitis viruses. No attempt is made to remove other plasma constituents.

Suomela attempts to achieve high purification of factor IX protein, but the product is obviously not free of all plasma

constituents. Thus, gel 4 in Figure 3 on page 150 is not completely free of high molecular material towards the top of the band. In isoelectric focusing (Figure 4), the peak is unsymmetrical (Figure 4A) or split (Figure 4B), indicating the presence of impurities. Since the even the highly purified product of Suomela is contaminated by its source, it is clear that the far less purified products of the other two references must be even more contaminated. The requirement in the present claims that the protein be free of poxvirus proteins further emphasizes that the claimed preparation is not intended to cover factor IX protein generated in tissue cells which have been infected by a vaccinia virus or like vector carrying the factor IX precursor gene.

The teachings of Suomela, Osterud and Schwinn will now be compared to the claimed features of the present invention.

(i) Plasma-free Preparation

Suomela, Osterud and Schwinn are all concerned with making factor IX from blood plasma sources. It is clear, therefore, that Suomela, Osterud and Schwinn do not teach or suggest a plasma-free preparation as now claimed in the present application.

(ii) Suitable for Human Use

Because the products of Suomela, Osterud and Schwinn are derived from plasma (and therefore are not plasma-free), the products of Suomela, Osterud and Schwinn suffer from the significant disadvantage of not being "risk-free" as far as

danger of contamination by the AIDS, virus or hepatitis virus is concerned. The plasma-free preparation of the present application, on the other hand, is "risk-free", which makes the presently claimed preparation patentably different from those taught by the cited references. Certainly, the "risk-free" nature of the product would be a differentiation from Suomela, Osterud and Schwinn which would be fully understood by the user of the product, i.e. a sufferer from hemophilia B. Such a patient would certainly regard the two types of product as different, since one could be fatal or inflict serious illness (i.e. the plasma-derived material), whereas the other (i.e. the present preparation) would not.

(iii) Recombinant DNA-Derived Factor IX Protein

The product of Suomela, Osterud and Schwinn has an active ingredient which is blood-derived factor IX protein. This is not the same as the claimed preparation of the present application which comprises, as active ingredient, recombinant DNA-derived factor IX protein.

(iv) Derived from a Single Human Individual and Essentially has the Amino Acid Sequence of Human Factor IX Protein

The product of Suomela, Osterud and Schwinn is derived from a plurality of source individuals. This is to be contrasted with the product of the present invention which is factor IX protein derived from a single human individual. In light of this, the

product of the present invention is monomorphic, whereas the product of Suomela, Osterud and Schwinn is polymorphic.

(v) Free from Contamination by Poxviruses and by all Plasma Constituents and has a Specific Activity of at least 90% of that Average Normal Human Plasma

As noted above, the presently claimed preparation is risk-free. This is not the case with products according to Suomela, Osterud and Schwinn, since those produces are derived from plasma.

In light of the above, it is believed that the claimed preparation would not have been obvious to a person of ordinary skill in view of the teachings of the references relied on by the Examiner. The only serious attempt to obtain highly purified factor IX is that reported by Suomela, and even there impurities remain in the product. Moreover, the three declarations of record in this case (one by Professor Brownlee, and the other two by independent experts, Dr. Tuddenaham and Dr. Gitschier), demonstraate the non-obviousness of the presently claimed preparation. The Examiner's position that those declarations only establish non-obviousness of the process is traversed. It is believed the declarations establish non-obviousness of all aspects of the claimed subject matter, i.e. the preparations per se, the method of treatment and the process of producing factor IX. The latter aspect is the subject of copending application serial number 07/231,671, filed August 12, 1988 and currently pending before Examiner Crouch.

The Brownlee declaration notes the poor expectations of success in research which ultimately led to the present invention. The reasons for these poor expectations resided in (1) the nature of the modifications which the factor IX precursor must undergo after translating and (2) the fact that tissue cells rather than live animals had to be used for the research. It was noted by Professor Brownlee that he was particularly doubtful about achieving the beta-hydroxylation (in any kind of cells) and about whether gamma-carboxylation would be achieved in tissue cells. Although a cell line H-4-11-Ec3 known to secret prothrombin was chosen, it did not necessarily follow (1) that this cell line would be capable of the gamma-carboxylation required in the modification of factor IX precursor or (2) that it would carry out beta-hydroxylation.

The Tuddenham declaration notes the complication of the post-translational modifications and the fact that he regarded the research leading to this invention as "highly speculative". Dr. Gitschier states that success was by no means assured.

It is clear, therefore, that success could not have been expected by a person of ordinary skill, and that Suomela, Osterud and Schwinn do not lead to the present invention. Withdrawal of the obviousness rejection based on those references is therefore respectfully requested.

Claims 29 through 32 have been rejected under 35 USC 103 as unpatentable over Davie when taken in view of Wood and further

in view of Schwinn. That rejection is traversed for the following reasons.

The patent to Davie relates to the isolation of DNA sequences for human factor IX. As noted by the Examiner, Davie does not teach or suggest that it is possible to utilize recombinant means in order to produce factor IX protein. Davie does indicate that a possible use of the isolated sequences is as a probe for detecting mutations in patients (column 9 lines 62-67), but does not suggest the expression of biologically active human factor IX protein.

It is also of interest to note that the Davie teaching appears to contain an inaccuracy with respect to the isolation of DNA sequences for factor IX. Comparison of Figure 1 of Davie with the structure elucidated by Anson et al, EMBO J., 3, 1053-1060 (1984), to which specific reference is made on page 10 lines 22-26 of the present application, indicates that Davie includes an alanine codon (see Figure 1 bases 907-909) which is not included in the structure as determined by Anson et al. A copy of page 1054 of the Anson paper is attached to this response for comparison purposes.

The Davie teaching is therefore deficient with respect to the presently claimed operation in two respects namely, first, with respect to the technical inaccuracy regarding the isolated DNA sequence and, secondly, as regards to the absence of any mention of expression and any suggestion that it is possible to make factor IX protein by recombinant DNA means.

Even if an attempt was made to produce recombinant factor IX using the Davie teaching (this is not suggested by Davie), the resulting expressed factor IX would be inactive because of the sequencing inaccuracy noted above. Any preparation containing that product would thus be useless for treating patients suffering from factor IX deficiency.

The above-noted deficiencies of Davie are not cured by Wood. Wood is concerned solely with factor VIII which is completely different from factor IX, and is irrelevant so far as the expression of factor IX is concerned. At the time of the present invention, it was known that factor IX expression, unlike factor VIII, involves at least two stages of protein processing involving peptide cleavage, as well as three distinct types of post-translational modifications, before secretion into the bloodstream as a 415 amino acid long mature biologically active glycoprotein. As discussed on page 2 of the present application, beginning at line 6, the post-translational modifications are the vitamin K-dependent carboxylation of 12-glutamic acid residues, the addition of several carbohydrate residues and the beta-hydroxylation of a single aspartic acid residue. The first two modifications are known to be required for activity. In addition, the prepeptide and the propeptide sequences totalling 46 amino acids have been removed as part of the processing of the precursor to give the mature active protein. Because of the complex and specialized nature of the processing and these modifications, it seemed likely at the date

of the present invention that the expression of active factor IX derived from factor IX DNA clones would present great problems and that one or more of the post-translational modifications would likely fail in any attempt to express recombinant factor IX.

The above concerns were not academic. Thus, Busby et al , Nature 316, 271-273 (1985) referred to on page 4 lines 19-21 of the present application, attempted the same system as used by Wood for factor IX and only succeeded in obtaining about 50% active factor IX.

It is believed, therefore, that the plasma-free factor IX preparation as claimed in the present application would not have been obvious to a person of ordinary skill in light of the Davie and Wood teachings. Wood focuses on factor VIII, and the Busby experiments discussed above only succeeded in obtaining about 50% active factor IX. Thus, the Examiner is incorrect in stating at page 5 lines 11-13 that "the factor IX produced by the hampster cells would inherently produce biologically active factor IX".

Referring to paragraph 5 beginning on page 5 of the official action, the Examiner's point concerning monomorphism has been discussed above. The factor IX protein is derived from a single human individual and not a plurality of source individuals. As a result, the product of the present invention is monomorphic. The important point here is the fact that the monomorphism of the product is a physical manifestation

(together with the "plasma-free" feature) of the important advantage that the product of the invention is "risk-free" so far as contamination by dangerous virus materials is concerned.

The Examiner's comments regarding the three executed declarations have been considered earlier. It is believed that those declarations, together with the claim language and supporting argumentation clearly establish non-obviousness of all aspects of the claimed invention, including the preparations per se, the method of treatment and the process of preparing factor IX. Withdrawal of all of the outstanding rejections is therefore believed to be very much in order. Such action is requested.

In the circumstances, it is believed that this application is now in a form suitable for immediate allowance and early action to that effect is requested.

Respectfully submitted,

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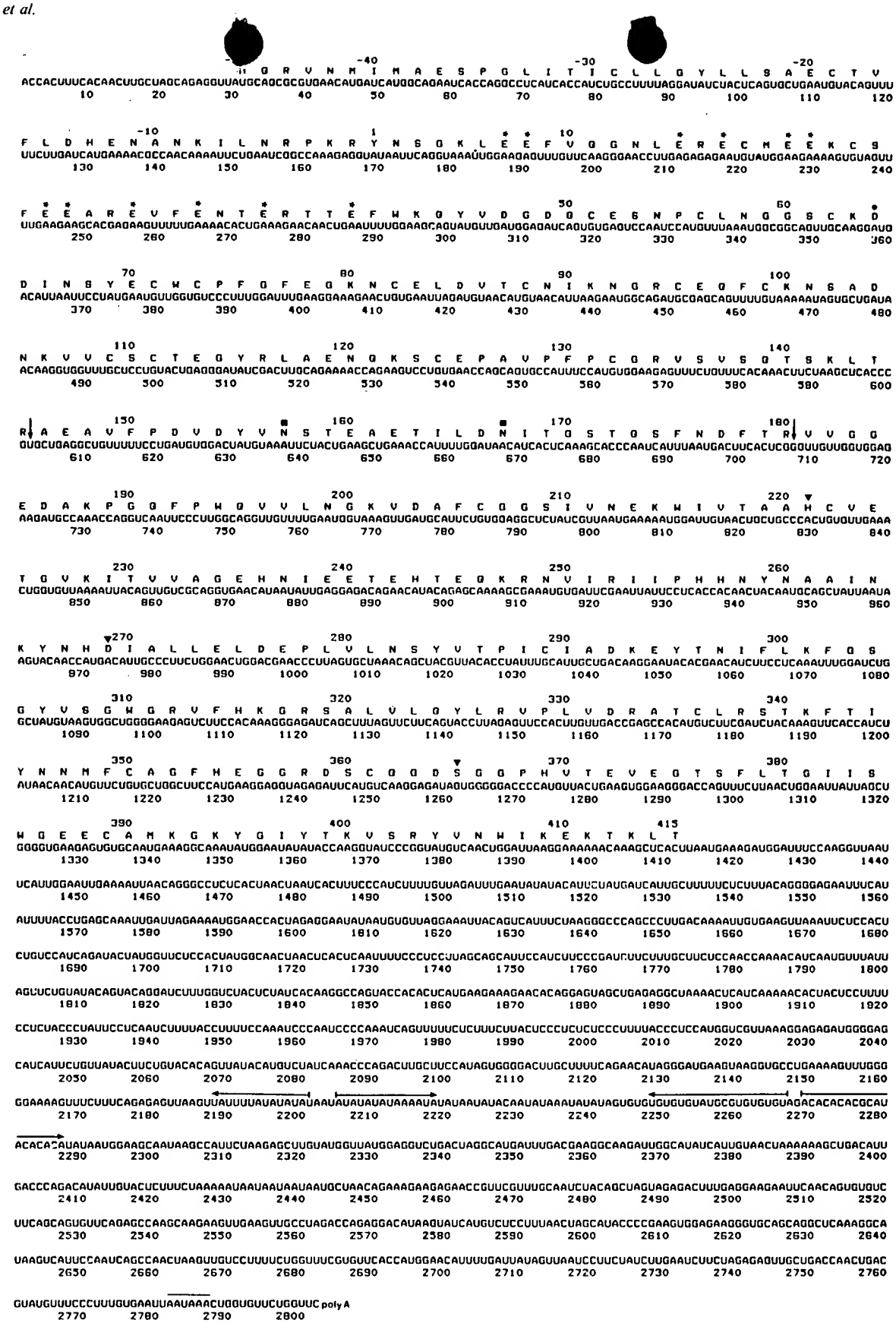

Fig. 2. Sequence of factor IX mRNA and its encoded protein. The symbols 1 – 415 define the mature protein and −46 to −1 the precursor region. The latter may be further subdivided into a hydrophobic signal region −46 to −21, and a hydrophilic precursor region −20 to −1 containing three basic amino acids between residues −4 to −1. Vertical arrows indicate the peptide bonds cleaved during activation in clotting. Post-translational modifications are marked (* = 12 γ-carboxyglutamyl residues, ● = β-hydroxyaspartyl and ■ = two Asn-linked carbohydrate residues). The AAUAAA consensus sequence is overlined. His (221), Asp (269) and Ser (365) are marked (▼). Local potential hairpin loops are shown by horizontal arrows.